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Human oocytes express ATP-sensitive K⁺ channels

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BACKGROUND: ATP-sensitive K⁺ (K_{ATP}) channels link intracellular metabolism with membrane excitability and play crucial roles in cellular physiology and protection. The K_{ATP} channel protein complex is composed of pore forming, Kir6.x (Kir6.1 or Kir6.2) and regulatory, SURx (SUR2A, SUR2B or SUR1), subunits that associate in different combinations. The objective of this study was to determine whether mammalian oocytes (human, bovine, porcine) express K_{ATP} channels.

METHODS: Supernumerary human oocytes at different stages of maturation were obtained from patients undergoing assisted conception treatments. Bovine and porcine oocytes in the germinal vesicle (GV) stage were obtained by aspirating antral follicles from abattoir-derived ovaries. The presence of mRNA for K_{ATP} channel subunits was determined using real-time RT–PCR with primers specific for Kir6.2, Kir6.1, SUR1, SUR2A and SUR2B. To assess whether functional K_{ATP} channels are present in human oocytes, traditional and perforated patch whole cell electrophysiology and immunoprecipitation/western blotting were used.

RESULTS: Real-time PCR revealed that mRNA for Kir6.1, Kir6.2, SUR2A and SUR2B, but not SUR1, were present in human oocytes of different stages. Only SUR2B and Kir6.2 mRNAs were detected in GV stage bovine and porcine oocytes. Immunoprecipitation with SUR2 antibody and western blotting with Kir6.1 antibody identified bands corresponding to these subunits in human oocytes. In human oocytes, 2,4-dinitrophenol (400 μM), a metabolic inhibitor known to decrease intracellular ATP and activate K_{ATP} channels, increased whole cell K⁺ current. On the other hand, K⁺ current induced by low intracellular ATP was inhibited by extracellular glibenclamide (30 μM), an oral anti-diabetic known to block the opening of K_{ATP} channels.

CONCLUSIONS: In conclusion, mammalian oocytes express K_{ATP} channels. This opens a new avenue of research into the complex relationship between metabolism and membrane excitability in oocytes under different conditions, including conception.

Key words: oocytes / ATP-sensitive K⁺ channels / Kir6.1/2 / SUR2A/B / membrane potential

Introduction

ATP-sensitive K⁺ (K_{ATP}) channels were originally described in cardiomyocytes (Noma, 1983), where they perform a cardioprotective role during metabolic stress. Physiological (high) levels of intracellular ATP maintain the channels in a closed state. However, upon a fall in intracellular ATP concentration, such as during hypoxia, the channels are released from inhibition, causing hyperpolarization of membrane potential. This hyperpolarization inhibits voltage operated Ca²⁺ channels and prevents intracellular Ca²⁺ overload (reviewed by Jovanović and Jovanović, 2009). K_{ATP} channels are also expressed in pancreatic β-cells and smooth muscle cells,

where they play crucial physiological roles. Pancreatic K_{ATP} channels control insulin secretion, whereas in smooth muscle cells they regulate muscular tone.

Structurally, K_{ATP} channels are composed of pore-forming inward rectifiers, Kir6.1 or Kir6.2, and regulatory, ATP-binding subunits, SUR1, SUR2A or SUR2B. It is generally accepted that four Kir6.x and four SURx are physically associated with each other to form a functional K_{ATP} channel. The properties of these channels are different in various tissues due to the combinations of the subunits forming the channel. SUR1 and Kir6.2 form the pancreatic type of K_{ATP} channels, SUR2A and Kir6.2 form the cardiac type of K_{ATP} channels, and SUR2B and Kir6.1 form the vascular smooth muscle type of K_{ATP} channels and

others (reviewed by Nichols, 2006). There are studies suggesting that further diversity may be generated by a combination of more than one type of Kir6.x and SURx subunits within an individual channel (Shyng and Nichols, 1997; Cui *et al.*, 2001; Chan *et al.*, 2008).

In any tissue where expressed, K_{ATP} channels regulate membrane potential and, by regulating membrane potential, regulate intracellular Ca²⁺ levels (reviewed by Nichols, 2006). It is well established that dynamics of the intracellular Ca²⁺ in oocytes play a crucial role in fertilization and embryo development, including oocyte maturation. Research so far has been focused on intracellular factors and signalling molecules that regulate intracellular Ca²⁺ dynamics, but there has been little focus on the membrane events (reviewed by Halet *et al.*, 2003; Jones, 2005; Machaca, 2007). K_{ATP} channels couple intracellular metabolism with membrane excitability and intracellular Ca²⁺ signalling (reviewed by Nichols, 2006). The presence of K_{ATP} channels in oocytes could play crucial roles in oocyte function, including fertilization and early embryonic events, and may provide protection during metabolic stress. Surprisingly, their presence in mammalian oocytes has never been elucidated. Here we describe the first step towards a better understanding of K_{ATP} channels in mammalian (including human) oocytes, demonstrating both the expression of Kir6.x and SURx subunits and the presence of functional, ATP-regulated K⁺ conductances in these cells.

Materials and Methods

Human, bovine and porcine oocytes

Supernumerary human oocytes that would otherwise have been discarded as a part of routine assisted conception treatments were used in these experiments. The oocytes were at different stages of maturity, namely germinal vesicle stage (GV), metaphase I (MI) and metaphase II (MII). Oocytes were collected from healthy women undergoing assisted reproduction treatment at Ninewells Assisted Conception Unit, Dundee, Scotland (HFEA centre # 0004). Ethical approval was provided by NHS Tayside Research Ethics committee (number 08/S1402/23).

Oocytes were donated for research at GV stage, MI and MII, and had been kept in culture in the clinical programme for varying lengths of time to comply with the constraints of the clinical programme. GV oocytes were characterized by the presence of the GV. MI oocytes had no GV and no polar body. MII oocytes were characterized by the presence of the first polar body. In ICSI treatment cycles, following injection of the mature MII oocytes, the uninjected immature oocytes were donated for research on Day 0, Day 1 or up to Day 5. By the time of use they may have matured further *in vitro*. Oocytes which had been inseminated but had failed to fertilize in both IVF and ICSI treatment cycles were donated to research on Day 2 to Day 5, which is a minimum of 24 and a maximum of 96 h in culture following assessment of fertilization. These were a mixture of GV, MI and MII; some of them had further matured *in vitro*. From the point of assessment of fertilization to being donated for research, all oocytes were cultured in 10 µl drops of cleavage medium under oil at 37°C in an atmosphere of 6% CO₂ in air. Oocytes which had failed to fertilize were defined as those showing no pronuclei and no cleavage.

Bovine and porcine oocytes were obtained by aspirating follicular fluid from large antral follicles of abattoir derived ovaries. Oocyte–cumulus complexes were retrieved and placed in Leibovitz medium containing 600 mIU/ml of hyaluronidase (Sigma Chemicals) to remove cumulus cells. Denuded GV intact oocytes were washed three times before being snap frozen for RNA extraction.

Real-time RT–PCR

For real-time RT–PCR, total RNA was extracted from pooled oocytes (12 human oocytes in different maturation stages, ~35 bovine oocytes or ~35 porcine oocytes all GV intact) using Absolutely RNA Nanoprep kit (Stratagene, La Jolla, CA, USA) and RNeasy plus Micro Kit (Qiagen, Crawley, UK) for human and bovine/porcine oocytes respectively, according to the manufacturer's instructions. RNA from human skeletal muscle, which is the tissue expressing all five subunits of K_{ATP} channels (Jovanović *et al.*, 2008), was used as a positive control (RNA was prepared as described in Jovanović *et al.*, 2008). For bovine and porcine oocytes, the positive control was RNA from heart embryonic H9C2 cells; these cells also express all five K_{ATP} channel subunits (Jovanović *et al.*, 2009a,b). For human oocytes, the specific primers for human K_{ATP} channel subunits (SUR2A, Kir6.2, SUR2B, SUR1 and Kir6.1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; as a control gene) were the same ones as previously described (Jovanović *et al.*, 2008). For bovine and porcine oocytes, the full sequences of K_{ATP} channel subunits are not yet known, so primers were designed taking into account the most conservative regions of subunits sequences in mammals with known K_{ATP} channel subunits sequences. The primers were as follows: for SUR1, sense, 5'-TCACACCGCTGTTCTGCT-3', antisense, 5'-AGAAGGAGCGAG GACTTGCC-3', 412 bp was the size of the PCR product; for SUR2A, sense, 5'-CTGGCTTTCTTCAGAATGGT-3', antisense, 5'-AAATACCC TCAGAAAAGACTAAAAC-3', 500 bp was the size of the PCR product; for SUR2AB (SUR2A+SUR2B together), sense, 5'-CATTGCC TACTTATTTCTCTCAG-3', antisense, 5'-ACCATTCTGAAGAAAGC CAG-3', 474 bp was the size of the PCR product; for Kir6.1, sense, 5'-CTGGCTGCTCTTCGCTATC-3', antisense, 5'-AGAATCAAACCG TGATGGC-3', 234 bp was the size of the PCR product; for Kir6.2, sense, 5'-CCAAGAAAGGCAACTGCAACG-3', antisense, 5'-ATGCTT GCTGAAGATGAGGGT-3', 449 bp was the size of the PCR product; for GAPDH, sense, 5'-GTCTTCACCACCATGGAGAA-3', antisense, 5'-TTCACCACCTTCTTGATGTCA-3', 488 bp was the size of the PCR product. Reverse transcription and whole transcriptome amplification for individual samples were achieved with the QuantiTect whole transcriptome kit (Qiagen, Valencia, CA, USA). RNA was first transcribed to cDNA using T-Script reverse transcriptase, and then the cDNA was ligated using a high-efficiency ligation mix and then amplified using REPLI-g. The resulting cDNA was used as a template for real-time PCR. An SYBR Green I system was used for the RT–PCR and the 25-µl reaction mixture contained: 12.5 µl of iQ™ SYBR® Green Supermix (2 ×), 7.5 nM each primers, 9 µl of ddH₂O and 2 µl of cDNA. In principle, the thermal cycling conditions were as follows: an initial denaturation at 95°C for 3 min, followed by 40 cycles of 10 s of denaturing at 95°C, 15 s of annealing at 56°C and 30 s of extension at 72°C. This protocol was modified for the m-lactic dehydrogenase-, GAPDH- and CK-specific primers changing the extension temperature to 55°C. The real-time PCR was performed in the same wells of a 96-well plate in the iCycler iQ™ Multicolor Real-Time Detection System (BioRad, Hercules, CA, USA). Data were collected following each cycle and displayed graphically (iCycler iQ™ Real-time Detection System Software, version 3.0A, BioRad). Primers were tested for their ability to produce no signal in negative controls by dimer formation and then with regard to the efficiency of the PCR. Efficiency is evaluated by the slope of the regression curve obtained with several dilutions of the cDNA template. Melting curve analysis tested the specificity of primers. Threshold cycle values, PCR efficiency (examined by serially diluting the template cDNA and performing PCR under these conditions) and PCR specificity (by constructing the melting curve) were determined by the same software. Each cDNA sample was duplicated; the corresponding no-RT mRNA sample was included as a negative control (blank).

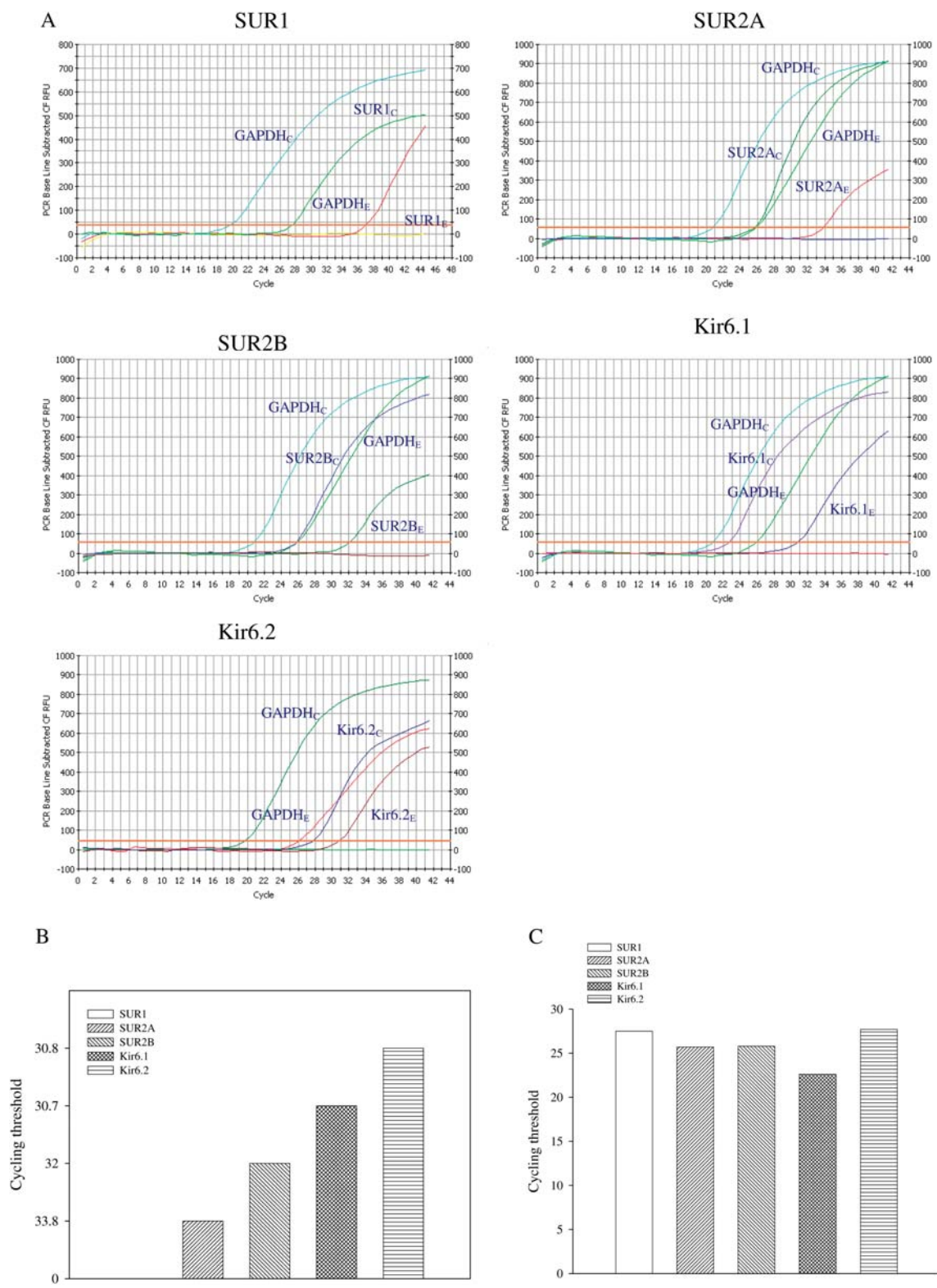


Figure 1 K_{ATP} channel subunits mRNAs in human oocytes. **(A)** Original progress curves for the real-time PCR amplification of SUR1, SUR2A, SUR2B, Kir6.1 and Kir6.2 cDNA in human oocytes. Curves labelled with subscripts C and E correspond to control and oocyte curves respectively. **(B and C)** Bar graphs showing cycle threshold for the real-time PCR amplification of SUR1, SUR2A, SUR2B, Kir6.1 and Kir6.2 from human oocytes (B) and human skeletal muscle (C) that served as a positive control. Absence of a bar pattern in graphs that is depicted in symbols means that no PCR product was obtained for this particular gene.

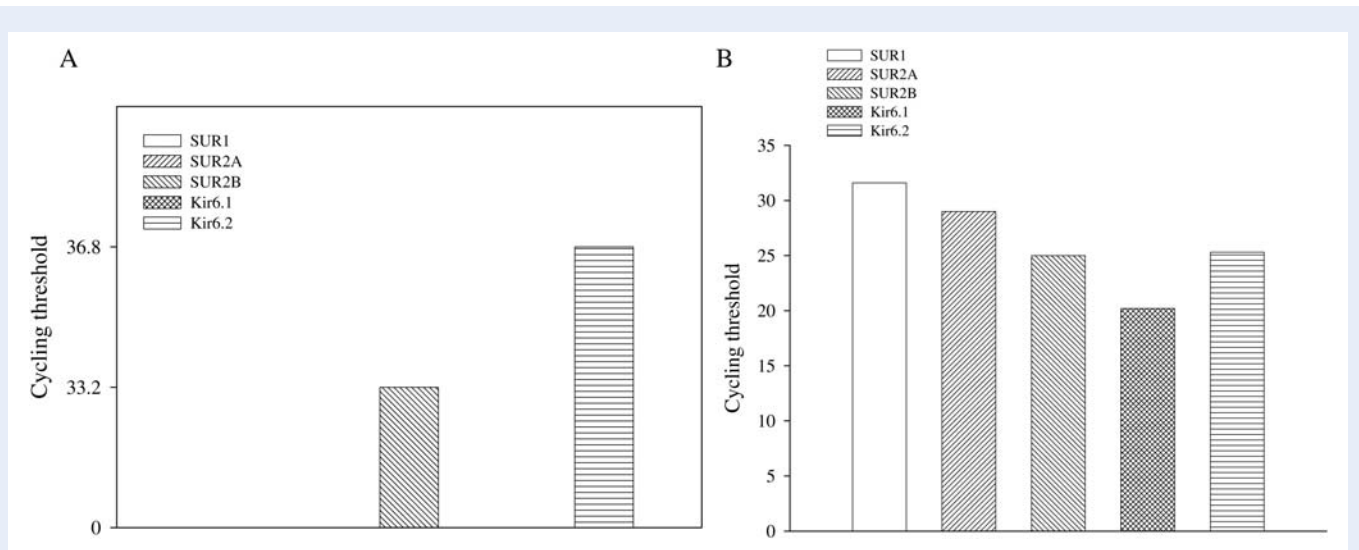


Figure 2 K_{ATP} channel subunits mRNAs in bovine oocytes. Bar graphs showing cycle threshold for the real-time PCR amplification of SUR1, SUR2A, SUR2B, Kir6.1 and Kir6.2 from bovine oocytes (A) and H9C2 cells (B) that served as a positive control. Absence of a bar pattern in graphs that are depicted in symbols means that no PCR product was obtained for this particular gene.

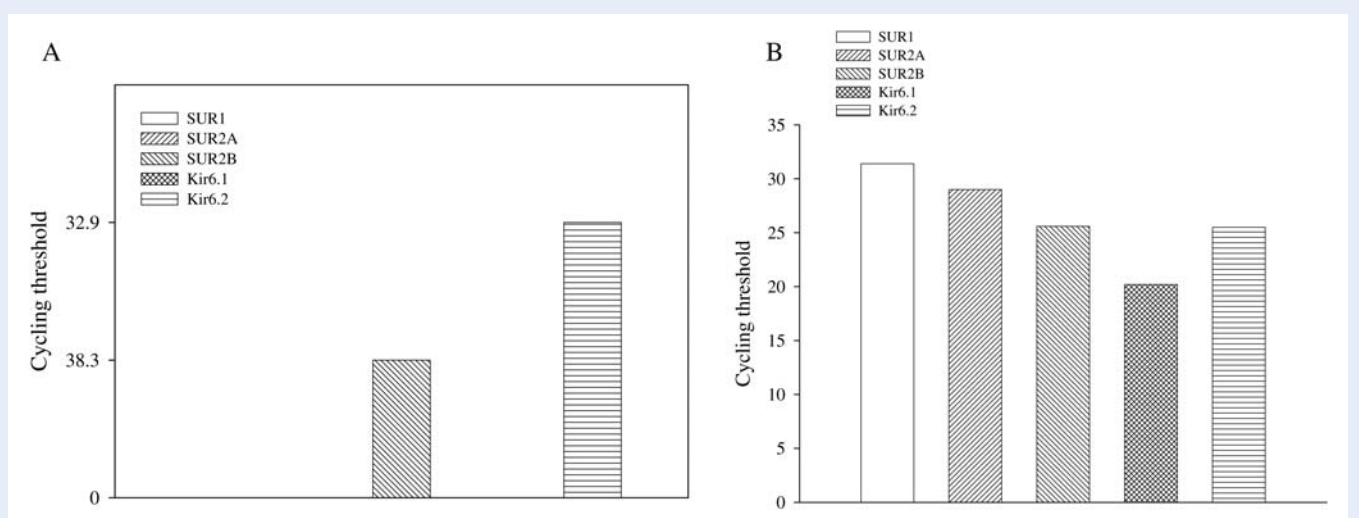


Figure 3 K_{ATP} channel subunits mRNAs in porcine oocytes. Bar graphs showing cycle threshold for the real-time PCR amplification of SUR1, SUR2A, SUR2B, Kir6.1 and Kir6.2 from porcine oocytes (A) and H9C2 cells (B) that served as a positive control. Absence of a bar pattern in graphs that are depicted in symbols means that no PCR product was obtained for this particular gene.

Immunoprecipitation/western blotting

Sheep anti-SUR2 and anti-Kir6.1 antibodies were used for immunoprecipitation and western blotting. To obtain sufficient amounts of oocyte proteins, we pooled 87 human oocytes (in GV, MI and MII stages) in buffer containing 1% Triton X-100 (w/w), 5 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA and SigmaFAST-TM protease inhibitor cocktail. The buffer was concentrated using Vivaspin 500 columns (Sartorius Stedium Biotech). Ten micrograms of the epitope-specific SUR2 antibody (an antibody that recognizes both SUR2A and SUR2B subunits) were pre-bound to Protein-G Sepharose beads and used to immunoprecipitate from protein extract. The pellets of this precipitation were run on SDS polyacrylamide gels for western analysis. Western blot probing was performed using 1/500 dilution of anti-Kir6.1 antibody and detection was achieved

using Protein-G HRP and ECL reagents. Cells that do not natively express K_{ATP} channels (A549 cells; Crawford *et al.*, 2002b; Jovanović *et al.*, 2003) were processed as described for oocytes and used as a control.

Patch clamp electrophysiology

Before patch clamp experiments, the zona pellucida was removed as described in Lai *et al.* (1994). In total, seven oocytes were patched. Experiments were performed while oocytes were immersed in Tyrode solution (in mM: 136.5 NaCl; 5.4 KCl; 1.8 CaCl₂; 0.53 MgCl₂; 5.5 glucose; 5.5 HEPES-NaOH; pH 7.4). For standard whole cell recording (where pipette solution replaces the cytosol), pipettes (resistance 3–5 MΩ) were filled with (in mM): KCl 140, MgCl₂ 1, ATP 0.01, EGTA-KOH 5,

HEPES–KOH 5 (pH 7.3). For experiments with 2,4-dinitrophenol (DNP), we used the perforated patch clamp electrophysiology (where the intracellular milieu remains intact). For this method the same pipette solution was used except with ATP omitted and amphotericin B (Sigma, 240 $\mu\text{g}/\text{ml}$) added. The membrane potential was normally held at -40 mV and the currents were evoked by a series of 400 ms depolarizing and hyperpolarizing current steps (-100 mV to $+80$ mV in 20 mV steps) recorded directly to hard disk using an Axopatch-200B amplifier, Digidata-1321 interface and pClamp8 software (Axon Instruments, Inc., Foster City, CA, USA).

Statistical analysis

Data obtained by patch clamp electrophysiology are presented as mean \pm SEM, with n representing the number of experiments. Mean values were compared using the paired or unpaired t -test, where appropriate. $P < 0.05$ was considered statistically significant.

Results

mRNA of K_{ATP} channel subunits in human, bovine and porcine oocytes

We have examined the presence of K_{ATP} channel subunits in human, bovine and porcine oocytes using real-time RT–PCR. In each run, there were positive controls with RNA from either human skeletal muscle (for human oocytes) or H9C2 cells (for bovine and porcine oocytes) and the technical soundness of the experiments was verified by the presence of GAPDH mRNA. In human oocytes, real-time RT–PCR revealed that SUR1 is not expressed whereas SUR2A, SUR2B, Kir6.1 and Kir6.2 mRNAs were detected in human oocytes (Fig. 1). In bovine GV stage oocytes, it was found that SUR2B and Kir6.2 subunits are expressed, but there was no evidence of the presence of SUR1, SUR2A and Kir6.1 (Fig. 2). Similar findings were obtained with porcine GV stage oocytes (Fig. 3).

Kir6.1 protein is associated with SUR2A/SUR2B proteins in human oocytes

SUR2A/B immunoprecipitate was obtained from pooled human oocytes. Western blotting of this precipitation with anti-Kir6.1 antibody has revealed a signal that was just below 50 kDa (Fig. 4). This signal was absent in A549 cells that do not natively express K_{ATP} channels. The size of Kir6.1 is 47 kDa, and as such the signal is where it is expected for this subunit, showing both that Kir6.1 protein is expressed and that it is associated with SUR2A/B subunits, consistent with the formation of functional K_{ATP} channels.

Whole cell K^+ current in human oocytes

To confirm the presence of functional K_{ATP} channels in human oocytes, we have performed patch clamp experiments. K_{ATP} channels are activated by a decrease in intracellular ATP levels. Using the perforated patch clamp technique, where the oocytes are dependent upon endogenous generation of ATP, we exposed oocytes to 400 μM of DNP, a metabolic inhibitor known to decrease intracellular ATP and activate K_{ATP} channels (Jovanović et al., 2009a,b). DNP (400 μM) increased the whole cell K^+ current (Fig. 5A, $n = 4$). The difference between K^+ currents in the presence and absence of DNP (400 μM) was statistically significant (Fig. 5A1; current at

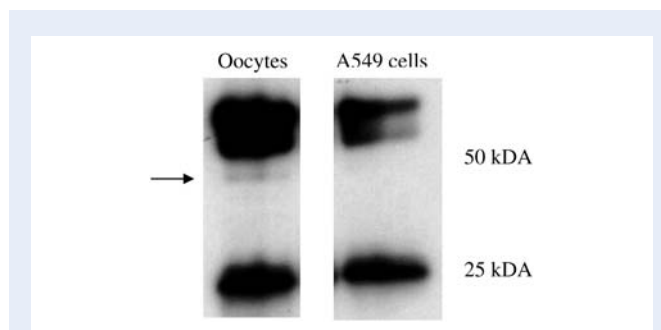
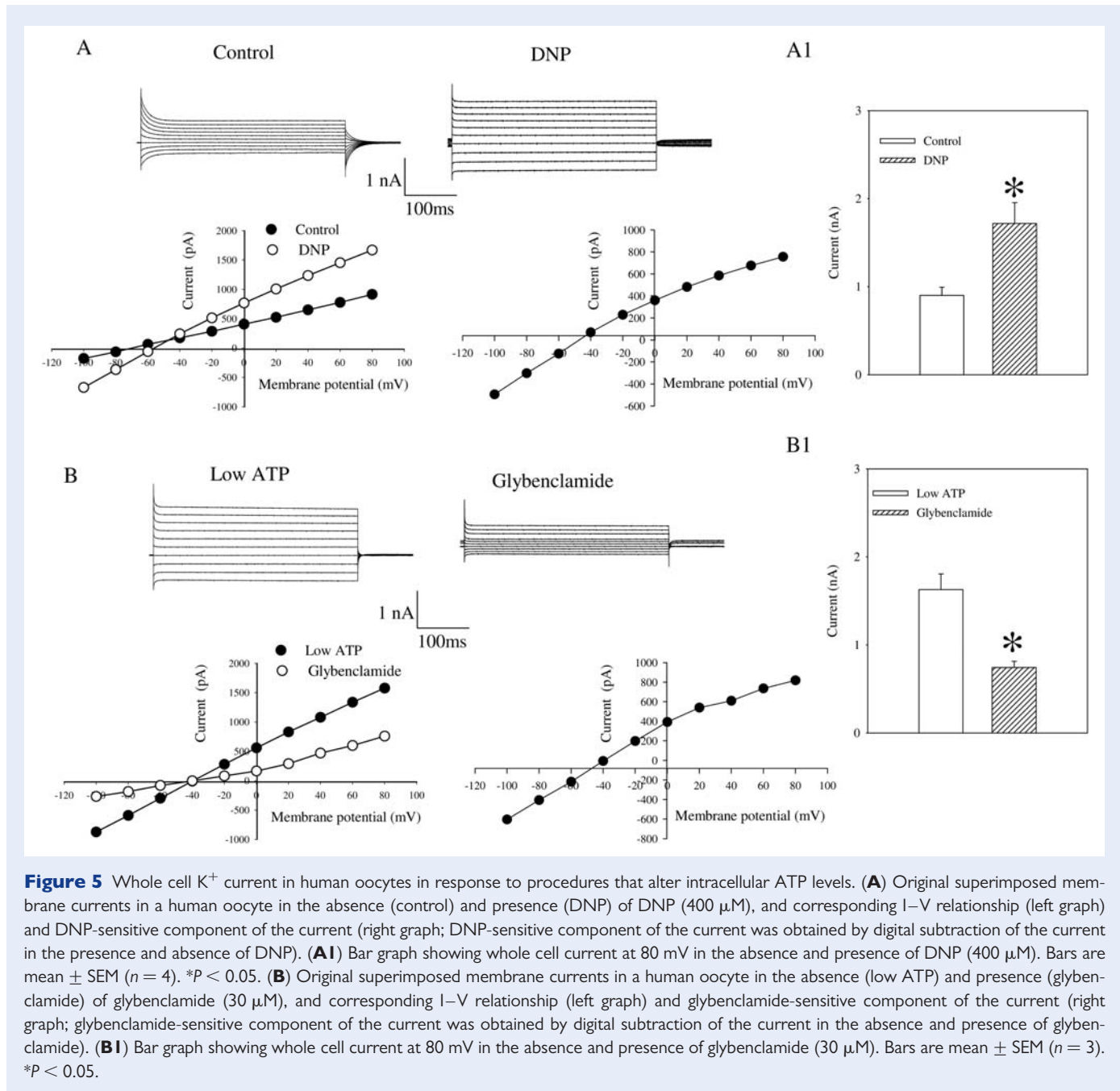


Figure 4 Kir6.1 protein that physically associates with SUR2A and/or SUR2B in human oocytes. A western blot of anti-SUR2 immunoprecipitate from human oocytes and A549 cells (cells that do not express K_{ATP} channels natively) with anti-Kir6.1 antibody under depicted conditions. The arrow indicates a signal corresponding to Kir6.1.

80 mV was 901 ± 93 pA in the absence and 1718 ± 236 pA in the presence of DNP, $P = 0.04$, $n = 4$). We further examined the effect of reduced cytoplasmic (ATP) by using standard whole cell recording (conditions where the pipette saline replaces the cytoplasm) with a pipette solution containing only 10 μM ATP. This low concentration of ATP does not inhibit K_{ATP} channel opening, but is sufficient to prevent a channel ‘run-down’. The current recorded under these conditions (current at 80 mV = 1628 ± 178 pA; $n = 3$) was significantly greater than the current recorded by the perforated patch clamp technique, when intracellular ATP was at its physiological level (current at 80 mV = 901 ± 93 pA; $n = 4$, $P = 0.01$; Fig. 5). This whole cell K^+ current observed with low intracellular ATP was inhibited by extracellular glybenclamide (30 μM), an oral antidiabetic known to block the opening of K_{ATP} channels (Fig. 5B; $n = 3$, $P = 0.01$).

Discussion

Human oocytes express four out of five known K_{ATP} channel subunits, strongly suggesting that human oocytes express K_{ATP} channels. K_{ATP} transcripts are also present in oocytes of other mammalian species and in human oocytes the Kir6.1 protein is present and is associated with SUR2A/B (demonstrated by immunoprecipitation/western blotting). The presence in human oocytes of transcripts encoding more subunits than required to form functional K_{ATP} channels in a single cell type is not unusual. In cardiomyocytes, there is mRNA of all five K_{ATP} channel subunits (Du et al., 2006). Originally, it was suggested that SUR2A and Kir6.2 alone form K_{ATP} channels in these cells (Inagaki et al., 1996), but more recent research would suggest that all of the expressed subunits could be a part of sarcolemmal K_{ATP} channels (Cui et al., 2001; Chan et al., 2008). The presence of mRNA of four subunits in human oocytes could indicate that K_{ATP} channels in these cells are composed of more than just a single SURx and Kir6.x subunit type. Since human oocytes at different stages of maturation were pooled, the presence of mRNAs for four subunits may reflect a change in the level of expression of different K_{ATP} channel subunits in oocytes during maturation. This is consistent with the findings that pure populations of bovine and porcine GV oocytes expressed only SUR2B and Kir6.2 mRNAs. These results could imply that K_{ATP} channels in mammalian



oocytes change their composition from the SUR2B/Kir6.2 combination at the GV stage (as these subunits were detected in all three species that provided GV oocytes) into SUR2A/Kir6.1 (as these subunits are detected only in human oocytes where MI and MII oocytes were present). Such changes in K_{ATP} channel subunit expression occur in response to changing physiological conditions; e.g. in ischaemically stressed heart cells there is up-regulation of Kir6.1, which apparently replaces Kir6.2 in the sarcolemmal K_{ATP} channel protein complex (Akao *et al.*, 1997). The electrophysiological and regulatory properties of SUR2B/Kir6.2 and SUR2A/Kir6.1 channel types are different (reviewed by Nichols, 2006) and changing expression would have effects on the membrane potential and intracellular Ca²⁺ as the oocyte matures (summarized in Fig. 6).

To date, the presence of transcripts for K_{ATP} channel regulatory and pore-forming subunits has always been associated with the presence of functional K_{ATP} channels in the plasma membrane (Akrouh *et al.*, 2009). We have used immunoprecipitation/western blotting to show that there is a population of Kir6.1 proteins that physically associate with SUR2A and/or SUR2B which are present in human oocytes, as this methodology is suitable to assess the presence of fully assembled K_{ATP} channels in human tissue (Jovanović *et al.*, 2008). To confirm the functional status of such channels in human oocytes, we applied patch clamp electrophysiology. The defining attribute of K_{ATP} channels is their regulation by intracellular ATP levels (Noma, 1983). It is well established that DNP, an uncoupler of oxidative phosphorylation, activates K_{ATP} channels in all cell types

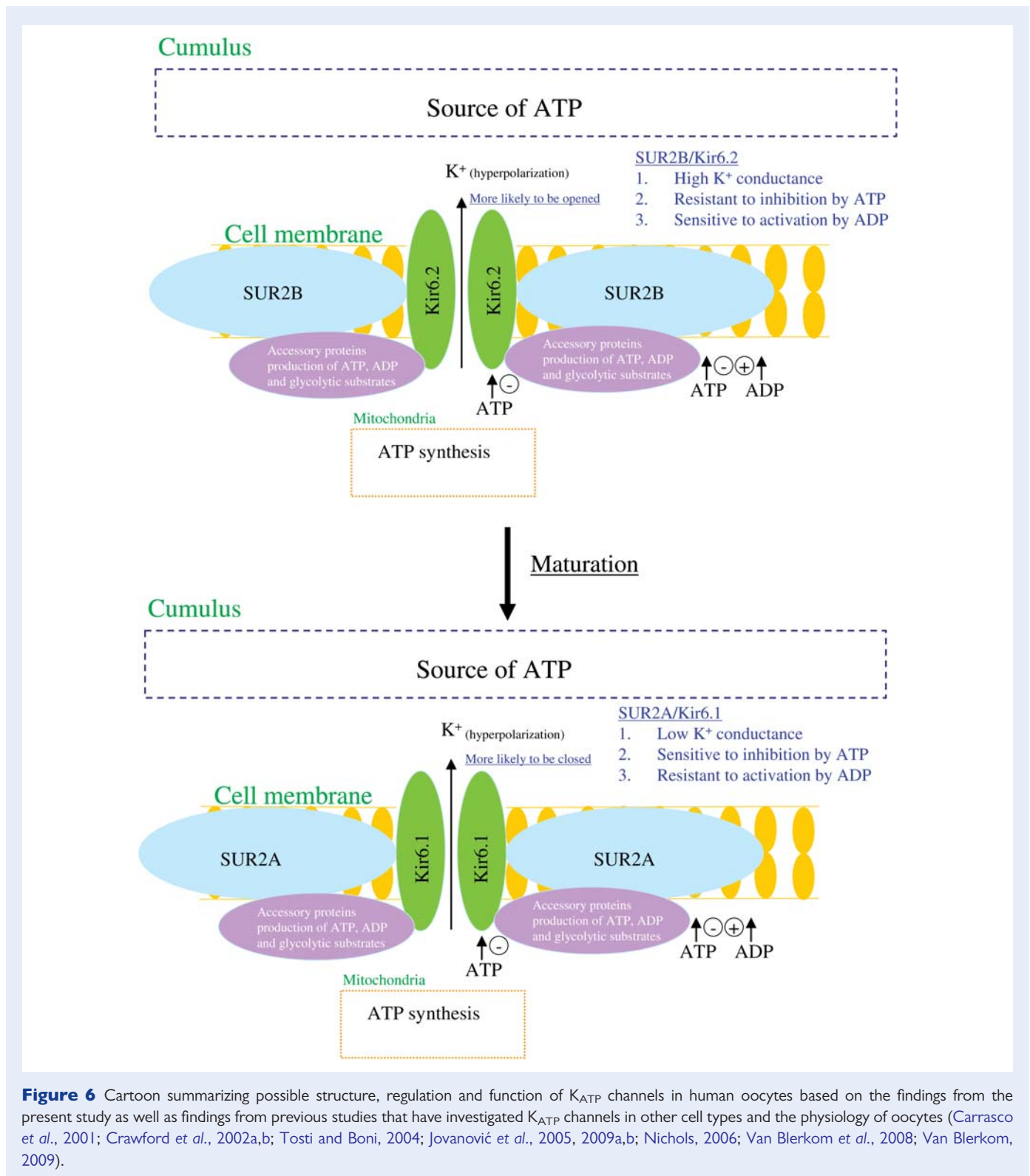


Figure 6 Cartoon summarizing possible structure, regulation and function of K_{ATP} channels in human oocytes based on the findings from the present study as well as findings from previous studies that have investigated K_{ATP} channels in other cell types and the physiology of oocytes (Carrasco et al., 2001; Crawford et al., 2002a,b; Tosti and Boni, 2004; Jovanović et al., 2005, 2009a,b; Nichols, 2006; Van Blerkom et al., 2008; Van Blerkom, 2009).

tested so far (Jovanović and Jovanović, 2001a,b; Jovanović et al., 2009a). It is also well known that the oral antidiabetic drug glybenclamide inhibits the activity of all known types of K_{ATP} channel (reviewed by Akrouh et al., 2009). In the present study, DNP induced a large, whole cell current which reversed close to E_K , which is a typical

finding when cells express K_{ATP} channels (Jovanović et al., 2009b). In cells without K_{ATP} channels or where the channel protein complex has been disrupted, DNP does not have this effect (Jovanović and Jovanović, 2001a; Du et al., 2010). Maintenance of low intracellular ATP by using the whole cell patch clamp configuration (pipette

saline containing 10 μ M ATP) was also associated with increased whole cell K⁺ current. This current was glybenclamide-sensitive, further supporting the notion that functional K_{ATP} channels are present in the plasma membrane of human oocytes. Both DNP-induced and glybenclamide-sensitive K⁺ currents exhibited a reversal potential consistent with K⁺ conductance and showed weak inward rectification (Fig. 5), a property typical of K_{ATP} channels. Our results, using different, but complementary techniques, suggest that oocytes express functional K_{ATP} channels in the plasma membrane.

As this is the first report to uncover the presence of K_{ATP} channels in oocytes, their possible role is still elusive. In some tissues, K_{ATP} channels are physiologically open, while in others they are normally closed and are activated by a specific signalling pathway (reviewed by Minimi *et al.*, 2004). In cell types where K_{ATP} channels are physiologically closed, their opening is associated with clamping membrane potential at the value of K⁺ equilibrium and counteracting membrane depolarization. In turn, this prevents an increase in intracellular Ca²⁺ if it is driven by Ca²⁺ influx via L-type Ca²⁺ channels (Jovanović and Jovanović, 2001a,b). On the other hand, in cell types where K_{ATP} channels are physiologically open, inhibition of these channels induces membrane depolarization, Ca²⁺ influx and increase in intracellular Ca²⁺. At the moment, we cannot draw a definite conclusion whether K_{ATP} channels are normally closed or open in oocytes, but the increase of K⁺ current by DNP would favour the idea that these channels were closed in the cell population we tested. In oocytes, the dynamics of intracellular Ca²⁺ concentration is crucial for regulation of oocyte maturation and fertilization (review by Tosti and Boni, 2004). Oocyte development and maturation in mammals are associated with changes in membrane potential and Na⁺ and K⁺ permeance ratio; the membrane potential of human and other mammals immature oocytes is approximately −30 to 40 mV and is suggested to decrease to a potential of −15 to 25 mV as the maturation process progresses (Eusebi *et al.*, 1984; Feichtinger *et al.*, 1988; Mattioli *et al.*, 1990; Emery *et al.*, 2001; Boni *et al.*, 2008). At fertilization, an increase of intracellular Ca²⁺ has been reported which is associated with membrane hyperpolarization. As Ca²⁺ oscillations are synchronized with episodes of membrane hyperpolarization, it has been suggested that Ca²⁺-sensitive K⁺ channels are responsible for this phenomenon (Miyazaki and Igusa, 1982; Dale *et al.*, 1996; reviewed by Tosti and Boni, 2004). The membrane potential of oocytes was shown to be regulated by cumulus that also provides ATP to oocytes and regulates polarity of suboolemmal mitochondria (Albertini *et al.*, 2001; Emery *et al.*, 2001; Van Blerkom *et al.*, 2008; reviewed by Van Blerkom, 2009). It is therefore quite possible that there is a link between ATP levels and regulation of membrane potential in oocytes. If this is the case, it is logical to think that K_{ATP} channels could provide this link. The physical proximity of mitochondria as a source of ATP and K_{ATP} channels is probably crucial in regulating K_{ATP} channels activity, as it has been shown that the level of K_{ATP} channel ligands surrounding the channel is much more important for the channel activity than the overall intracellular concentration of these ligands (Elvir-Mairena *et al.*, 1996). Suboolemmal mitochondria could regulate the probability of the channel opening by feeding ATP into the K_{ATP} channel protein complex that, besides channel subunits, contain enzymes utilizing ATP as a substrate. Specifically, K_{ATP} channel subunits physically associate with adenylate kinase, creatine kinase and five glycolytic enzymes, and substrates of these enzymes and their

products (ATP, ADP, AMP, lactate, 1,3-bisphosphoglycerate) serve as K_{ATP} channel ligands that can both open and close the channel (Carrasco *et al.*, 2001; Crawford *et al.*, 2002a,b; Jovanović and Jovanović, 2005; Jovanović *et al.*, 2005). At the same time, the K_{ATP} channel protein complex can also produce ATP itself (Jovanović *et al.*, 2009a,b; Du *et al.*, 2010) and provide substrates for suboolemmal mitochondria (Fig. 6). Thus, K_{ATP} channels could transduce metabolic changes during maturation and fertilization into changes in membrane excitability and intracellular Ca²⁺ dynamics.

Conclusion

In conclusion, this study has shown that human, bovine and porcine oocytes express K_{ATP} channels. The property of these channels to transduce metabolic changes into changes in membrane excitability supports the notion that K_{ATP} channels could play important roles in oocyte maturation and fertilization.

Authors' roles

All authors have contributed to the study design, revised and approved the manuscript. In addition to that, E.B., E.D., K.M.W., V.K., M.M. and E.E.T. were involved in oocyte collection, while Q.D., S.J. and A.S. have performed experiments using these oocytes. A.J. has designed and supervised the study, drafted and approved the manuscript.

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